NOV 0 2 2001

Oey, Jan

From: Kinser, Robin D.
Sent: Friday, November 02, 2001 11:36 AM
To: 'Jill Schultz'
Cc: Kinser, Robin D.; Oey, Jan; Robert A. Libby (E-mail)
Subject: RE: Nicotine Glucuronide validation data

Dear Jill--

You are authorized to proceed with analysis of the glucuronides of nicotine and its metabolites , based on the interim validation data below. I am authorizing you to proceed despite the failure of nicotine glucuronide in freeze/thaw and room temperature stability testing, largely because the cotinine glucuronide did pass short term stability testing, and my memory of previous indications of a systematic nicotine contaminant. I am concerned, however, about this apparent nicotine contamination in the SPE cartridges, and the impact this may have on our measurements of nicotine and metabolites in non-smokers.

After Mark has returned and re-grouped, let's plan a discussion call to identify the questions which have arisen during validation and begin preparing action plans to address those issues.

–Robin

-----Original Message-----
From: Jill Schultz [SMTP:jill.schultz@Covance.Com]
Sent: Friday, October 19, 2001 10:01 AM
To: Holt.Klausvon@pmintl.ch; kuhl.peter@pmintl.ch; rustemeier.Klaus@pmintl.ch; Schepers.Georg@pmintl.ch; Stabbert.Regina@pmintl.ch; tricker.anthony@pmintl.ch; Hans-Juergen.Roethig@pmusa.com; Jan.Oey@pmusa.com; robin.d.kinser@pmusa.com
Subject: Nicotine Glucuronide validation data

Attached are the tabulated validation data for the glucuronide assay and summary sheets. These data are not 100% QC checked at this stage, hence they may be subject to minor change. Please review and contact me with any questions or your authorization to proceed with sample analysis.

Mark had the following comments to add regarding these data:

Water blank samples containing internal standard (technically the zero calibration standard which is not used in the regression) for nicotine typically contains an amount equivalent to between 20-40% of the LLOQ, which was also apparent during the aglycone validation. Normal practice would advocate raising the LLOQ for this analyte to a level whereby this interference is always less than 20%. However, on a within batch basis, such interference has been very consistent, reflected by the fact that acceptable precision and accuracy has been achieved at all levels down to the LLOQ of 1 ng/mL. It is suspected that background levels for nicotine derive, at least in part, from the solid phase extraction sorbent. I would recommend that the LLOQ remain at 1 ng/mL, this phenomenon being discussed within the validation reports.

Dilution QCs were diluted with control urine instead of water, as stated in the protocol, prior to analysis. This does not affect the

data, however an amendment will be required to document this protocol deviation.

Intra and inter-day data would suggest that acceptance limits of 20% (rather than 15%) for precision and accuracy of low QC samples during sample analysis may be more realistic for the glucuronides...for consideration by PM.

Stability data demonstrate that the glucuronides are stable stored at room temperature for 24 h and subjected to 3 additional freeze/thaw cycles. However, the apparent percent aglycone liberated by nicotine glucuronide 2 ng/mL QC samples taken through the SPE method (referenced against deconjugated 2 ng/mL QCs), using peak area ratios, was in the region of 33%. I believe this to be an artefact due to

quantifying area ratios below the LLOQ, compounded by the issue raised in the first paragraph above. If degradation was occurring, noticeable aglycone concentrations would be expected from glucuronide QCs prepared at high concentrations (not apparent).

Stock solution stability will be assessed when solutions are prepared for the sample analysis phase, to save on expensive standards.

Please note that Mark will be on vacation 10/23 through 11/11. Phil Turpin will serve as his backup while he is away.

Regards,

Jill

<< File: nicotine.xls >> << File: cotinine.xls >> << File: pmgluc~1.xls >>